DEVELOPMENT OF A NOVEL RADIATION IMAGING DETECTOR SYSTEM FOR *IN VIVO* GENE IMAGING IN SMALL ANIMAL STUDIES

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Many studies in developmental molecular biology deal with following the expression of a gene at different stages of an organism's development. Presently *in situ* hybridization and immunochemical assays are available to follow the gene expression in a developing organism at single moments in time for one organism. One must sacrifice the organism to make a measurement, essentially taking a snap shot of the state of expression of the gene of interest. Progress will be reported on a new type of gene imaging technology which takes advantage of the emission properties of the radioisotope iodine 125 (^{125}I) as the gene probe and utilizes crystal scintillators and a position sensitive photomultiplier tube. Iodine 125 decays via electron capture emitting a 35 keV gamma-ray with the prompt emission of several 27-32 keV Kα and Kβ shell X-rays. Because of this a coincidence condition can be set to detect the ^{125}I decays thus reducing background radiation contribution to the image.

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Summary

We will report progress on a new type of gene imaging technology which takes advantage of the emission properties of the radioisotope iodine 125 as the gene probe and utilizes crystal scintillators and a position sensitive photomultiplier tube.

Introduction

There are only a few methods used to follow gene expression in live animals i.e. *in vivo*. A technique recently reported was used to identify live transgenic animals used green fluorescent fusion proteins (GFP) to identify live transgenic zebra fish (Peters et al., [1]). A transgenic zebra fish carrying a transgene which expresses GFP which could then be detected using UV illumination. The GFP method only works for transparent animals.

When a gene is expressed it first results in the transcription of an mRNA transcript which is then translated into a protein. Anti-sense mRNA probes have been constructed that could be introduced into a live organism. Dewanjee et al., [2, 3 , 4] has shown that it is possible to construct and successfully use radiolabeled anti-sense mRNA probes to detect the target mRNA *in vivo* in mice using a standard gamma camera as the detector system. Dewanjee et al. injected ^{111}In (171.2 keV) radiolabeled antisense and sense mRNA probes into mammary tumor-bearing BALB/c mice. They observed high concentration of the probe in the mammary region of the mouse and concluded that the antisense probe could be used for noninvasive imaging of c-*myc* oncogene mRNA for malignant tumors. The gamma camera system they used produced scintiphotographs with a resolution of the order of 0.5 cm.

Clearly higher position resolution is needed to further study this technique.

Priliminary Results

We are testing the usefulness of radioactive iodine 125 (^{125}I) as the radioactive substance linked to a probe to follow the progression of gene expression in a live organism. This will be accomplished by using standard probes linked to the isotope but detected and imaged with a novel type of radiation imaging detector. The radioisotope ^{125}I is commonly used in molecular biology and medical research and is readily available linked to nucleic acids and antibodies from companies providing probes for gene research[5]. One can easily construct a DNA, RNA or monoclonal antibody probe labeled with ^{125}I.

An important aspect of the imaging technique is the choice of the radioisotope. Iodine 125 has a half life of ~60 days and decays via electron capture with the emission of a 35 keV gamma-ray with the prompt emission of several 27-32 keV Kα and Kβ shell X-rays from the daughter product ^{125}Te [6]. Because of these correlated phenomena a coincidence condition can be set to detect the ^{125}I decays and thus reducing background radiation. This coincidence technique has already been shown to be useful for the detection of extremely low concentrations (on the order of attomole) of ^{125}I linked to a probe[7]. We have constructed a position sensitive photomultiplier tube (PSPMT) based gamma-ray imaging detector made of a high resolution collimator and a thin YAP crystal scintillator coupled to a position sensitive photomultiplier tube (PSPMT) to test the usefulness coincidence mode imaging.

Experimental Setup

The Hamamatsu R2486 PSPMT was used to image gamma-ray detection with various scintillating crystals. The R2486 PSPMT has an active area of about 50 mm in diameter. It has an eleven stage proximity focused parallel dynode mesh structure and 16x16 crossed wire anodes with a pitch of 3.75 mm. Initial tests have been performed with a 60mm x 60mm x 1mm plate of YAP scintillator using a 250 nanoCurie ^{125}I calibration source. The detector arrangement is shown in figure 1.

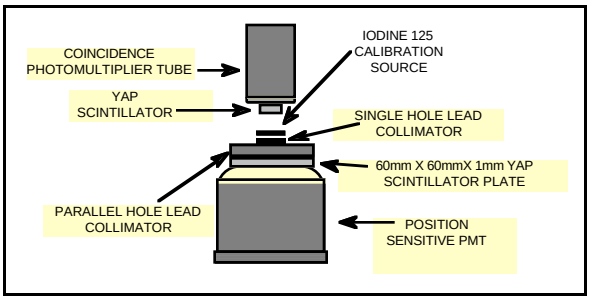

Figure 1. Detector arrangement for coincident detection of X-ray and gamma-ray emissions from ^{125}I.

Electronics and Data Acquisition System

To reduce the number of wires to be instrumented we have experimented with pairing anode wires to reduce the number of channels to instrument by a factor of two. We have found that there is only a slight decrease in position resolution by pairing up anode wires resulting in a 8 x 8 configuration. The data acquisition system is CAMAC based and tailored to the detector system and this particular research application. A Macintosh Power PC workstation is the host computer. All data acquisition and computer imaging is accomplished with the Kmax data acquisition software obtained from the Sparrow Corporation.

Determination of the position of interaction of the scintillator light on the photocathode of the PSPMT is achieved by an analog to digital conversion (ADC) of the signal on the anodes of the PSPMT. The electronic signal from the last dynode of the PSPMT is inverted and then passed through discriminator electronics to detect an event and determine if the pulse is above a desired threshold, and an output gate signal is generated. This gate signal is used as a trigger for the ADC measurement using a CAMAC based ADC for each of the paired anode.

Results

An example of an image obtained using 1 cm thick parallel hole lead collimator and a single 2 mm diameter hole lead collimator with this setup with coincidence mode enabled and disabled is shown in figure 2. The right image was obtained in coincidence with a 3 cm x 3 cm x 1 cm YAP detector placed on the other side of the source as shown. One can see that the background is considerable less in the coincident case.

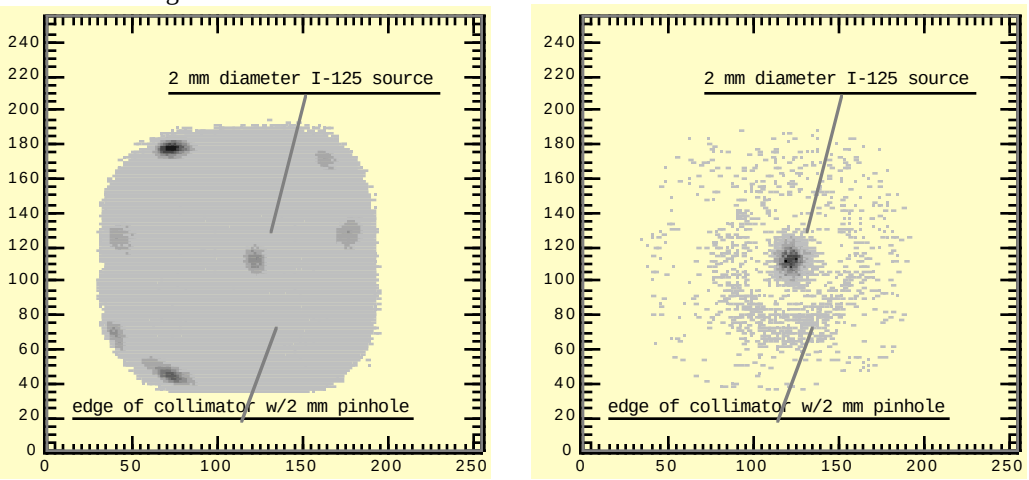

Figure 2. Images obtained using 1 cm thick parallel hole lead collimator and a single 2 mm diameter hole lead collimator coincidence mode off (left image) and then on (right)

Conclusion

Future work will be directed to achieving actual imaging studies using laboratory mice. Although a coincidence method has been used with ^{125}I to detect extremely low concentrations of a target molecule or compound, this has been shown to work only when the activity is quite localized, or for non-imaging applications. Our probes are going to be distributed across the whole body of the mouse with the desire to image "hot" spots; therefore good counting statistics is highly desirable. It is clear that this aspect of the imaging requires further study.

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